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ORIGINAL ARTICLE

Association analysis of SNP-63 and indel-19 variant in the calpain-10 gene with polycystic ovary syndrome in women of reproductive age☆

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CAPN10 variants;
Haplotypes;
Polycystic ovary syndrome

Abstract
Background: Polycystic ovary syndrome is a complex and heterogeneous disease leading to reproductive, as well as metabolic problems. It has been suggested that there may be a genetic predisposition in the aetiology of polycystic ovary syndrome. The identification of calpain 10 gene *(CAPN10)* as the first candidate gene for type 2 diabetes mellitus has focused the interest in investigating their possible connection with the polycystic ovary syndrome. This syndrome is associated with hyperinsulinaemia and insulin resistance, two metabolic abnormalities associated with type 2 diabetes mellitus.
Objective: To investigate if there is association between the SNP-63 and the genetic variant indel-19 of *CAPN10* gene and polycystic ovary syndrome in women of reproductive age.
Material and methods: This study included 101 women (55 with polycystic ovary syndrome and 46 without polycystic ovary syndrome). The genetic variant indel-19 was identified by electrophoresis of the amplified fragments by PCR, and the SNP-63 by PCR-RFLP.
Results: The allele and genotype frequencies of the two variants do not differ significantly between women with polycystic ovary syndrome and the control women group. The haplotype 21 (defined by the insertion allele of indel-19 variant and C allele of SNP-63) was found with higher frequency in both study groups, being more frequent in the polycystic ovary syndrome patients group, however, this difference was not statistically significant.

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Conclusions: The results suggest that SNP-63 and indel-19 variant of CAPN10 gene do not represent a risk factor for polycystic ovary syndrome in our patient group.

PALABRAS CLAVE
Gen *CAPN10*;
Haplotipos;
Síndrome de ovario poliquístico

Análisis de asociación del SNP-63 y la variante indel-19 del gen de calpaína-10 con síndrome de ovario poliquístico en mujeres en edad reproductiva

Resumen
Antecedentes: El síndrome de ovario poliquístico es una enfermedad compleja y heterogénea que implica problemas reproductivos y metabólicos. Se ha sugerido una predisposición genética en la etiología de este síndrome. La identificación del gen de la calpaína-10 *(CAPN10)* como el primer gen asociado a diabetes mellitus tipo 2 suscitó el interés por investigar su posible relación con el síndrome de ovario poliquístico, debido a que este síndrome se asocia a hiperinsulinemia y resistencia a la insulina, 2 anormalidades metabólicas relacionadas con diabetes.
Objetivo: Investigar si existe asociación entre el SNP-63 y la variante indel-19 del gen *CAPN10* y el síndrome de ovario poliquístico en mujeres en edad reproductiva.
Material y métodos: El estudio incluyó a 101 mujeres (55 con síndrome de ovario poliquístico y 46 clínicamente sanas). La variante indel-19 se identificó mediante corrimiento electroforético de los fragmentos amplificados por PCR y el SNP-63 por PCR-RFLP.
Resultados: Las frecuencias alélicas y genotípicas de las 2 variantes no difieren significativamente entre pacientes con síndrome de ovario poliquístico y mujeres del grupo control. El haplotipo 21 (definido por el alelo inserción de la variante indel-19 y el alelo C del SNP-63) se encontró con mayor frecuencia en los 2 grupos de estudio, siendo más frecuente en el grupo de pacientes; sin embargo, esta diferencia no fue estadísticamente significativa (p = 0.8353).
Conclusiones: Los resultados sugieren que el SNP-63 y la variante indel-19 del gen *CAPN10* no son factores de riesgo para síndrome de ovario poliquístico en nuestro grupo de pacientes.

Background

Polycystic ovary syndrome is a heterogeneous endocrine disorder, considered to be the most common cause of anovulation, hyperandrogenism and infertility in women of childbearing age[1]. Other major clinical characteristics of polycystic ovary syndrome also include obesity (mainly in the upper segment) and metabolic abnormalities, such as hyperinsulinaemia and insulin resistance, which is a risk factor for developing type 2 diabetes mellitus and cardiovascular disease[2,3].

The prevalence of polycystic ovary syndrome in different populations is 3-7% in women of childbearing age and 60-80% in women with hyperandrogenism[4]. In Mexico, a prevalence of 6% has been reported[5].

The physiopathology of polycystic ovary syndrome is complex. However, the 2 main hormonal changes observed in patients with polycystic ovary syndrome include increased circulating levels of the luteinising hormone and insulin (hyperinsulinaemia); in fact, it has been suggested that there is certain synergism between both, so ovarian hyperstimulation due to insulin would lead to hyperandrogenism[2]. Polycystic ovary syndrome has been considered a disorder with a genetic component in its aetiology, and this condition has been supported by pronounced familial aggregation and by the documentation of the fact that the degree of concordance is higher in monozygotic twins than in dizygotic twins[2]. The theories related to the inheritance mode of polycystic ovary syndrome include a multifactorial model where the interaction of environmental factors (nutrition and physical activity) and a group of causative genes (candidate susceptibility genes) contribute to its phenotypic variability[6].

The association between polycystic ovary syndrome with insulin resistance and the risk of developing type 2 diabetes mellitus led to the assumption that the primary defect causing this syndrome was connected to one of the mediators of the insulin response metabolic pathway[7]. The identification of the calpain-10 gene *(CAPN10)* by Horikawa et al.[8] in 2000 as the first gene related to type 2 diabetes mellitus triggered interest to investigate its possible relation with polycystic ovary syndrome, given the documented participation of the protein product of this gene in the insulin signalling pathway[6,7,9-11].

Calpain-10 is a non-lysosomal cysteine-protease activated by calcium and an atypical calpain, since it lacks IV domain[8,12]. This calpain can be seen in different tissues, such as pancreatic islets, the skeletal muscle, the liver and adipocytes[13]. Although its specific function has not been fully described yet, it has been suggested that calpain-10 is an important molecule for the function of pancreatic ß-cells, since it has been demonstrated to regulate the exocytosis of insulin secretory granules[14,15]. It has also been stated that calpain-10 facilitates the trans-

location of GLUT4 through a distal effect on the insulin action pathway. In addition, calpain-10 has also been involved in the reorganisation of the actin cytoskeleton related to both GLUT4 vesicle translocation and to insulin secretion[14,16].

The *CAPN10* gene is located in the distal region of the long arm of chromosome 2 (2q37.3) and it is 65,674 nucleotides long, which are distributed in 15 exons and 14 introns[14,16]. From the variants identified in the *CAPN10* gene, those that stand out are an SNP located in intron 3, the SNP-43 (G>A; rs3792267), and another one located in intron 13, the SNP-63 (C>T; rs5030952), as well as an insertion/deletion of 32 bp, referred to in the medical literature as SNP-19, found in intron 6 (indel-19 variant; rs3842570), since it has been described that, in an independent manner or as an haplotype, these contribute to the susceptibility to type 2 diabetes mellitus in several populations[8,17]. Some studies have focused on the relation between polycystic ovary syndrome and the variants in *CAPN10*[1,6,9-11,18-24]; nevertheless, the influence of genetic variations on the susceptibility to develop multifactorial pathologies, such as polycystic ovary syndrome, seems to vary among populations. Thus, the objective of this study was to analyse the distribution of alleles and genotypes of two of the most common variants of the *CAPN10* gene (the SNP-63 and the indel-19 variant) in patients with polycystic ovary syndrome and in women without polycystic ovary syndrome, so as to determine if there is a relation between this syndrome and the two variants in women of childbearing age.

Material and methods

The study involved a total of 101 women (55 diagnosed with polycystic ovary syndrome and 46 without polycystic ovary syndrome), all of whom were of childbearing age (18-38 years) and not related to each other. The sample sizes for the 2 study groups (55 and 46) are above the minimum (36 subjects) for a biallelic polymorphism system (α 0.05; p = 0.05), according to Chakraborty[25].

All the women included in the study were of mixed race and lived in the state of Jalisco. Prior to signing the Informed consent form for study participation, women who accepted to participate were informed about the purpose, the benefits and the procedures of the project. The study protocol was conducted pursuant to the Declaration of Helsinki and authorised by the Local Ethics and Research Committee of the Instituto Mexicano del Seguro Social (N. 2005/1/I/064).

Patients with polycystic ovary syndrome were subsequently recruited in the Fertility and Endocrinology departments of the Highly Specialised Medical Unit of the Gynaecology and Obstetrics Hospital of the National Medical Centre of the West of the Mexican Social Security Institute. Polycystic ovary syndrome was diagnosed in accordance with the Rotterdam ASRM/ESHRE consensus criteria, revised in 2003[26], accompanied by the comprehensive management of polycystic ovary syndrome (clinical practice guideline, CIE-10:E28, Mexican Social Security Institute)[27] (Table 1). Out of 55 patients with polycystic ovary syndrome, 44 were overweight (body mass index > 25) and 11 suffered from obesity (body mass index ≥ 30).

The women from the control group were randomly selected and had regular menstrual cycles between 21-28 days, with a body mass index < 25 and without personal or family history of polycystic ovary syndrome. They visited the hospital for a general medical examination.

The study did not include pregnant women or women under anovulatory, antiandrogen or corticosteroid therapy. Confusion variables, such as physical activity and diet, were not taken into account for this study.

Genotypification

The genomic DNA was obtained from peripheral blood leukocytes, according to a slightly modified standard protocol[28], and it was stored in Tris-EDTA buffer with pH 8.0, at -20°C until its processing.

To identify the SNP-63 (C16378T), a fragment of 192 bp was amplified and subjected to digestion with 2 U of the restriction enzyme *HhaI* at 37°C, according to the manufacturer's instructions (New England Biolabs, Ipswich, US). This resulted in 1 fragment of 192 bp in the presence of the T allele and 2 fragments, one of 162 bp and another one of 30 bp, in the presence of the C allele[29]. To identify the indel-19 variant, a fragment of 155 bp was amplified in the presence of deletion (3 repetitions of 32 bp, 2R allele), while a fragment of 187 bp was amplified in the presence of insertion (2 repetitions of 32 bp, 3R allele)[29] (Table 2). The difference in base pairs of CRP products and enzyme digestion products was analysed in 6% polyacrylamide gel, dyed with silver nitrate (Fig. 1). As an internal quality control, reference genotype samples were selected for confirmation by capillary electrophoresis in an automatic sequencer - Beckman-Coulter, CEQ8800 model (California, US).

The haplotypes formed by 2 polymorphisms of the *CAPN10* gene were inferred considering the indel-19 variant in the first position and SNP-63 in the second position. This results in 4 possible haplotypes: haplotype 11 (deletion allele of the indel-19 variant, C allele of the SNP-63), haplotype 12 (deletion allele of the indel-19 variant, T allele of the SNP-63), haplotype 21 (insertion allele of the indel-19 variant, C allele of the SNP-63) and haplotype 22 (insertion allele of the indel-19 variant, T allele of the SNP-63).

Statistical analysis

The allelic frequencies of the 2 variants of the *CAPN10* gene were determined using the direct counting method of observed genotypes and were presented as simple frequencies.

The comparison between allelic, genotypic and haplotype frequencies of the indel-19 variant and SNP-63 among patients with polycystic ovary syndrome and women from the control group, as well as the Hardy-Weinberg balance determination, were conducted using the chi-square test (X^2). The result was considered statistically significant if the probability value was lower than 0.05.

The data analysis was performed using the statistical programme RxC with 10,000 iterations[30]. The programme HaplotypeReconstructor_v0.6 was used to infer haplotypes.

Table 1 Revised Rotterdam ESHRE/ASRM 2003 criteria, accompanied by CPG CIE-10: E28.

Diagnostic criteria (2 out of 3 criteria)		
1. Oligo and/or anovulation	History of 8 or less menstrual cycles within a year or menstrual cycles shorter than 26 days or longer than 35 days and P4 < 4 ng/ml	
2. Signs of hyperandrogenism	Clinical	Hirsurtism[a] Acne Alopecia - male pattern
	Biochemical	Total testosterone > 84.7 ng/dl Free testosterone > 0.75 ng/dl Free testosterone/androgen index DHEA DHEAS > 2,459 ng/ml LH/FSH relation > 2 Lower levels of sexual-steroid transporting globulin
3. Polycystic ovaries	Presence of 12 or more follicles in each ovary with a diameter from 2 to 9 mm and/or increased ovarian volume > 10 mm (ultrasound assessment)	
Exclusion of other pathologies[b]		
Syndromes of severe insulin resistance		Cushing's syndrome HAIRAN Androgen secreting neoplasm
Congenital adrenal hyperplasia		Deficiency of 21 hydroxylase (17 hydroxyprogesterone 2-3 ng/ml)
Androgen secreting tumours		Sertoli-Leydig tumours, adrenal adenomas
Hyperprolactinaemia		Increased levels of prolactin
Hypogonadotropic hypogonadism		Low serum levels of FSH and reduced E2 levels
Premature ovarian failure		Increased serum levels of FSH and reduced E2 levels
Complementary studies for the assessment of comorbidities		
Fasting lipid profile		
Fasting serum glucose		
Fasting serum insulin		
Glucose/insulin ratio < 4.5 indicates insulin resistance		

DHEA: dehydroepiandrosterone; DHEAS: dehydroepiandrosterone sulphate; E2: estradiol; FSH: follicle-stimulating hormone; HAIRAN: hyperandrogenism, insulin resistance and acanthosis *nigricans* syndrome; LH/FSH: luteinising hormone/follicle-stimulating hormone ratio; P4: progesterone.

[a] Assessed using a standardised scoring system (Ferriman-Gallwey).

[b] The exclusion of other pathologies is part of the initial assessment. Revised Rotterdam ESHRE/ASRM 2003 criteria[26]. Comprehensive management of polycystic ovary syndrome[27].

Results

The average age of women from the control group was 26 years, and 80% of these women had menstrual cycles from 25 to 28 days. On the other hand, in patients with polycystic ovary syndrome, the average age was 29 years, and 88% of them had menstrual cycles from 30 to 60 days.

Table 3 shows the distribution of alleles and genotypes of SNP-63 and the indel-19 variant in patients with polycystic ovary syndrome and women from the control group. The distribution of observed genotypes of the 2 variants was in accordance with the expected results from the Hardy-Weinberg balance determination ($p > 0.05$). The genotypic frequencies of the indel-19 variant and SNP-63 show no significant differences among the group of patients with polycystic ovary syndrome and women from the control group ($p = 0.7240$ and $p = 0.6793$, respectively). The analysis of allelic frequencies showed that the insertion allele of the indel-19 variant and the C allele of the SNP-63 were more frequent in the group of patients with polycystic ovary syndrome compared to the control group. Nevertheless, this difference was not statistically significant ($p = 0.5587$ and $p = 0.5671$, respectively).

The 4 possible haplotypes were observed in the study population. The combination 21 (insertion allele of the indel-19 variant, C allele of the SNP-63) and the haplodiplotype 21/21 were more frequent in the 2 study groups (Table 4). However, when comparing the observed frequencies of the total haplotypes and haplodiplotypes between the group of patients with polycystic ovary syndrome and the control group, there were no statistically significant differences ($p = 0.8353$ and $p = 0.8231$, respectively).

In addition, the allelic frequencies of the indel-19 variant and SNP-63 observed in the studied population were compared with the frequencies described in other studies conducted in populations from America (Chile and Brazil),

Tabla 2 Abordaje metodológico para la identificación de las dos variantes del gen *CAPN10*.

Variante	Iniciadores	Fragmento amplificado	Enzima de restricción	Fragmentos esperados
SNP-63	Sentido 5'AAGGGGGGCCAGGGCCTGACGGGGGTGGCG3' Antisentido 5'AGCACTCCCAGCTCCTGATC3'	192 pb	*Hha*I GCG^C	Alelo C 162 +30 pb Alelo T 192 pb
Indel-19 inserción/ deleción de 32 pb	Sentido 5'GTTTGGTTCTCTTCAGCGTGGAG3 Antisentido 5'CATGAACCCTGGCAGGGTCTAAG3'	155 pb 187 pb	-	Alelo Ins 187 pb Alelo Del 155 pb

pb: pares de bases.

Asia (China, Korea, India and Turkey) and Europe (Germany, Spain and England) (Table 5).

Discussion

The association between polycystic ovary syndrome with insulin resistance and the risk for developing type 2 diabetes mellitus has been described. This study was conducted to assess the possible association between this syndrome and 2 polymorphisms of the *CAPN10* gene (indel-19 variant and SNP-63) involved in the risk haplotype (121) for type 2 diabetes mellitus in Mexican-Americans[8]. It was found that the distribution of alleles and genotypes was similar in patients with polycystic ovary syndrome and women without polycystic ovary syndrome, which makes it possible to suggest that, in our group of patients, the indel-19 variant and SNP-63 do not represent a risk factor for developing polycystic ovary syndrome. This finding is in accordance with the results from 2 previous studies, one conducted in England[9] and the other in Turkey[18], where, apart from analysing SNP-63 and the indel-19 variant, 2 other variants of the *CAPN10* gene were analysed (SNP-44 and SNP-43), and none of the 4 variants was associated with polycystic ovary syndrome. Though there are several studies that show the involvement of variants in the *CAPN10* gene in the development of the polycystic ovary syndrome, the association is not always made with the same variant, given that, for instance, in German women, this syndrome was associated with SNP-56[11]; in women from Spain[6], Turkey[19] and India[1], it was associated with SNP-44. Furthermore, in 2 populations of Latin America, Chile[20] and Brazil[21], the syndrome was associated with SNP-43.

There are relatively few studies that investigated the relation between polycystic ovary syndrome and the haplotypes of the *CAPN10* gene. In a study conducted in German women, in which 8 variants of the *CAPN10* gene were analysed (including indel-19 and SNP-63), it was found that haplotype TGA2AGCA represents a higher risk for polycystic ovary syndrome[11]. In women from India, where 5 variants were analysed (also including indel-19 and SNP-63), there was a significant association with haplotype 21121[1]. In Spanish women, where 4 variants were analysed (SNP-44, SNP-43, indel-19 and SNP-43), haplotype 1121 was

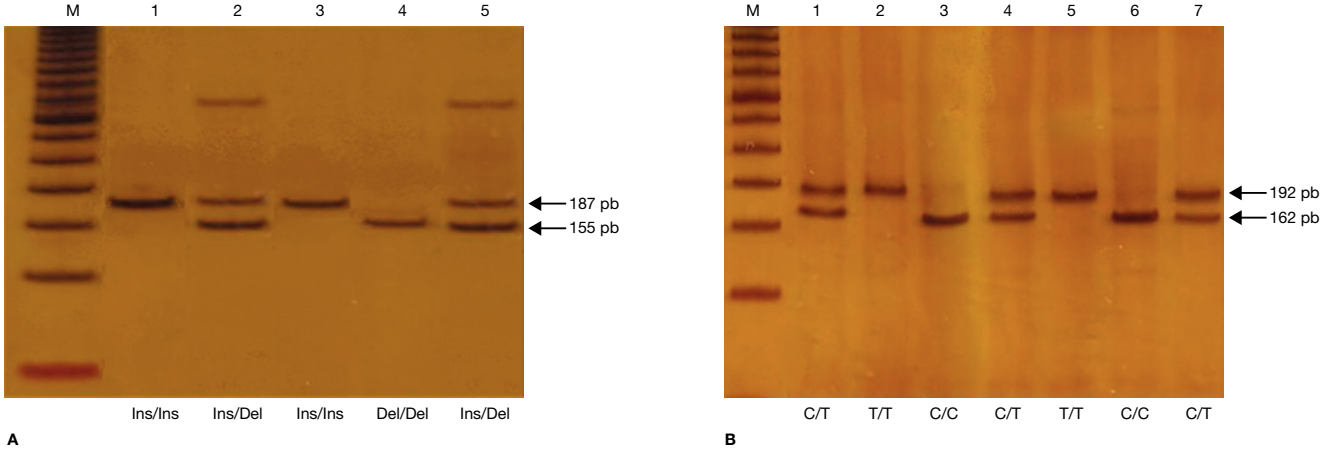

Fig. 1 A) Genotypification of the indel-19 variant. CRP products separated in 6% polyacrylamide gel, dyed with silver nitrate. M: molecular weight marker (DNA ladder of 50 bp). Lanes 1 and 3 homozygous Ins/Ins (fragment of 187 bp); lanes 2 and 5 heterozygous Ins/Del (fragments of 187 bp and 155 bp); lane 4 homozygous Del/Del (fragment of 155 bp). B) Genotypification of SNP-63. CRP products digested with the *Hha1* enzyme, separated in 6% polyacrylamide gel, dyed with silver nitrate. M: molecular weight marker (DNA ladder of 50 bp). Lanes 3 and 6 homozygous C/C (fragment of 162 bp); lanes 1, 4 and 7 heterozygous C/T (fragments of 192 bp and 162 bp); lanes 2 and 5 homozygous T/T (fragment of 192 bp).

Tabla 3 Distribución de alelos y genotipos del SNP-63 y la variante indel-19 en las pacientes con síndrome de ovario poliquístico y las mujeres del grupo control.

Polimorfismo			Pacientes con SOP n (%)	Controles n (%)	Valor de p	OR, IC del 95%
Indel-19	Genotipo	Ins/Ins	21 (38)	16 (35)	0.7240	0.831
		Del/Ins	27 (49)	22 (48)		
		Del/Del	7 (13)	8 (17)		
	Alelo	Ins	69 (63)	54 (59)	0.5587	-
		Del	41 (37)	38 (41)		
SNP-63	Genotipo	C/C	38 (69)	30 (65)	0.6793	0.821
		C/T	17 (37)	15 (33)		
		T/T	0 (0)	1 (2)		
	Alelo	C	93 (85)	75 (82)	0.5671	-
		T	17 (15)	17 (18)		

Del: deleción; n: número de sujetos de estudio (genotipo) o cromosomas (alelo); Ins: inserción; SOP: síndrome de ovario poliquístico.

Tabla 4 Distribución de haplotipos y diplotipos del SNP-63 y la variante indel-19 en pacientes con síndrome de ovario poliquístico y controles.

		Pacientes con SOP n = 110[a]		Grupo control n = 92[a]		p
		n	(%)	n	(%)	
Haplotipos	21	68	(62)	54	(59)	0.8353
	22	25	(23)	21	(23)	
	11	16	(15)	17	(18)	
	12	1	(1)	0	(0)	
		n = 55		n = 46		
Diplotipos	21/21	20	(36)	16	(35)	0.8231
	21/22	15	(27)	13	(28)	
	21/11	12	(22)	9	(20)	
	21/12	1	(2)	0	(0)	
	22/22	3	(5)	1	(2)	
	22/11	4	(7)	6	(13)	
	11/11	0	(0)	1	(2)	

SOP: síndrome de ovario poliquístico.
n: número de individuos; SOP: síndrome de ovario poliquístico.
[a] n: número de cromosomas.

associated with hypercholesterolemia in patients with polycystic ovary syndrome[10]. In Korean women, where only 3 variants were analysed (SNP-43, indel-19 and SNP-63), there was an association between polycystic ovary syndrome and haplotype 111[22]. In this study, since only 2 variants were analysed, the most frequent haplotype in patients with polycystic ovary syndrome was haplotype 21 (insertion allele of the indel-19 variant, C allele of SNP-63), which is partially similar to haplotype 121 described by Horikawa et al. in the Mexican-American population[8]. Nevertheless, no statistically significant value was obtained when comparing haplotype frequencies with the control group, so its possible association with polycystic ovary syndrome was discarded in our group of patients studied. Therefore, it is necessary to analyse other variants in the *CAPN10* gene to investigate the contribution of an extended haplotype to the development of this syndrome.

Although our results suggest the lack of involvement in the development of polycystic ovary syndrome of the indel-19 variant and SNP-63 of the *CAPN10* gene, both in an individual manner and as haplotype, this study is particularly important given that, to the best of our knowledge, it is the first study that investigates the possible association of variants of the *CAPN10* gene and haplotypes to polycystic ovary syndrome in the Mexican population.

The comparative analysis of allelic frequencies among the different populations demonstrated that allelic frequencies of the indel-19 variant in our group of patients with polycystic ovary syndrome were different from those found in patients with polycystic ovary syndrome of the Brazilian population[21]. Moreover, there were differences among the frequencies of our control group and the control group of the Indian population[1]. In relation to the SNP-63, there were also differences when comparing the observed allelic frequencies, both in our group of patients with polycystic ovary syndrome and in our control group, with the frequencies reported in India[1]. There were also differences with the frequencies reported in the study conducted in England[9] and the study conducted on the Spanish population, but in the latter, differences were only found among control groups[6]. Differences found in England and India may be due to the little or almost no documented migration of the English and Indians to Mexico. The analysis of several genetic polymorphisms in European and American populations, including Brazil, has shown that there is a variation in the genetic structure of different populations, possibly as a result of the various interethnic combinations[31-34]. Our results suggest that the Mexican mixed race population has particular genetic

Tabla 5 Frecuencias alélicas de la variante indel-19 y el SNP-63 del gen *CAPN10* en diferentes poblaciones.

			Ins/Del-19				SNP-63			
Continente	Población de estudio	Control/SOP	Alelo Ins		Alelo Del		Alelo C		Alelo T	
			Control	SOP	Control	SOP	Control	SOP	Control	SOP
América	Presente estudio[a]*	46/55	0.59	0.63	0.41	0.37	0.82	0.85	0.18	0.15
	Chilenos[20]	70/50	0.59	0.61	0.41	0.39	0.81	0.79	0.19	0.21
	Brasileños[21]	59	ND	0.37*	ND	0.63*	ND	0.87	ND	0.13
Asia	Hindúes[1]	298/248	0.45*	0.54	0.55*	0.46	0.95*	0.95*	0.05*	0.05*
	Turquía[18]	50/44	0.56	0.55	0.44	0.45	0.86	0.92	0.14	0.08
Europa	Inglaterra[9]	525/185	0.61	0.59	0.39	0.41	0.92*	0.92*	0.08*	0.08*
	Españoles[6]	92/55	0.65	0.53	0.35	0.47	0.92*	0.88	0.08*	0.12

ND: no disponible; SOP: síndrome de ovario poliquístico.
[a] Grupo de referencia.
* $p < 0.05$.

characteristics which make it different from other populations around the world.

The genetic factors involved in polycystic ovary syndrome and its various expressions could explain the phenotypic variation in the clinical presentation of the syndrome, which is physiologically heterogeneous and controversial for genetic determinants, given that each population has shown different results even when analysing the same polymorphism. The ethnic differences among study subjects seem to be contributing to these differences.

Therefore, due to the multifactorial aetiology of polycystic ovary syndrome, the combination of all the genetic risk factors should be considered the molecular mechanism of this syndrome, as the effect of only one gene is not enough to favour the development of the endocrine-metabolic imbalance presented by patients, together with triggering environmental factors that contribute to the onset of the syndrome.

Conclusions

The allelic, genotypic and haplotype distribution of the indel-19 variant and SNP-63 does not vary significantly among patients with polycystic ovary syndrome and women from the control group. Thus, these findings suggest that these 2 variants of the *CAPN10* gene do not represent a risk factor for polycystic ovary syndrome, so they cannot be used in the clinical practice as genetic risk markers in the studied population. The population analysis showed that there are differences between the Brazilian, Spanish, English and Indian population, which indicate that frequencies of the variants of the *CAPN10* gene are heterogeneous among different populations.

Conflict of interest

The authors declare that there are no conflicts of interest.

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